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SHORT REVIEW

Coffee drinking: The rationale for treating it as a potential effect modifier of carcinogenic exposures

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Abstract. Clinical and epidemiological studies on cancer etiology seldom treat coffee drinking as a potential effect modifier. Yet caffeine exerts significant effects upon a large variety of physiologic, cellular and molecular systems. Caffeine, 'the world's most popular drug', is also a fundamental research tool, widely used in clinical studies on drug metabolism, and in experimental studies on cell cycle checkpoints, DNA repair, and apoptosis, among many other. Caffeine can profoundly alter cell cycle checkpoint function and several mechanisms of DNA repair, as well as carcinogen metabolism. The impact of caffeine on cell cycle checkpoint function occurs in spite of it being nonmutagenic in traditional mutagenesis assays. A complex body of biologic evidence suggests that caffeine-containing beverages can both enhance and antagonise potentially carcinogenic exposures. However, most pathways leading to the ultimate effects in human beings remain unknown. It is unclear whether any of the hundreds of compounds contained in coffee and tea exert a direct and significant carcinogenic effect *per se* in any human tissue at usual conditions of use. Reasons exist to consider that coffee may sometimes be an indirect, positive confounder. The study of interactions between caffeine-containing beverages and environmental agents in well defined groups of healthy and diseased people could yield new insights into checkpoint signal transduction and other mechanisms of carcinogenesis. Information on the use of caffeine-containing beverages should more often be integrated in studies on the role of gene–environment interactions in the pathogenesis of cancer.

Key words: Caffeine, Cell cycle, Coffee/etiology, Coffee/genetics, Coffee/metabolism, Coffee/physiology, DNA repair, Epidemiology, Epidemiology/methods, Molecular, Mutation/genetics, Neoplasms/genetics

Abbreviations: ATM = mutated in ataxia telangiectasia; ATR = ataxia telangiectasia and Rad-3-related; CYP = cytochrome P450; EPC = exocrine pancreatic cancer; PAHs = polycyclic aromatic hydrocarbons; UV = ultraviolet

> Authors have a tendency to grow lyrical when discussing the effects of caffeine-containing beverages on man.
> Bengt A. Kihlman [1]

Introduction

It is rather uncommon to see an epidemiological study where coffee drinking is treated as a potential effect modifier. This is so in cancer epidemiology and in many other research areas, too. Perhaps this results from two joint processes: an unawareness of the evidence on the very rich metabolic, cellular and genetic effects of caffeine, and a conventional reluctance to analyze interactions if the rationale is not evident. Yet the rationale does often exist, and epidemiological thinking on coffee and other caffeine-containing beverages should become more coherent with current biologic knowledge.

A myriad of beverages, compounds, and biological effects

Awareness of the rich biological, clinical, and psychosocial effects of coffee, tea and other methylxanthine-containing beverages dates back to antiquity [1–4]. And since 1820 – when the structural formula of caffeine was established – progress in knowledge of the causes for such effects has never seemed to stop. Evolving from the field of organic chemistry to DNA research, studies on caffeine and molecular biology are nowadays closely interwoven [1, 5].

Research on the potential carcinogenic effects of coffee and on the influence that coffee may have on

the carcinogenic effects of other exposures shows a remarkable diversity of nuances, and a discernible, logical trend towards the molecular and genetic levels of analysis [1, 5–11] (Table 1).

As we will see, excellent reviews on the biologic effects of caffeine and other coffee compounds are available. Thus, we shall here draw attention to just a few lines of research and findings that in our view are particularly relevant for clinical and epidemiological research on cancer. Indeed, the main object of this assay is the carcinogenic process; however, the general points are also applicable to other areas. Although several hundred volatile and nonvolatile compounds have been identified in roasted and in

Table 1. A diversity of statements on the carcinogenicity of coffee compounds

Kihlman [1]	There is hardly any other drug that affects the genetic material in so many different ways as caffeine. It not only produces mutations and chromosomal aberrations, but also strongly enhances the lethal, mutagenic and chromosome-damaging effects of other agents. The potentiating effects of caffeine are likely to be the result of its ability to inhibit repair of the damage caused by the other agents to chromosomal DNA (Preface). Caffeine is a purine derivative and thus related to adenine and guanine, which are key components of DNA and RNAs. Adenine is also a component of many coenzymes and of adenosine triphosphate (ATP), which is fundamental in exchange of energy (Preface). The risk of point mutations being produced by caffeine in man is practically nonexistent. The risk of chromosome aberrations being produced in man by caffeine is negligible. No conclusive evidence can be found in the literature for caffeine being a carcinogenic agent (pp. 412–413). Caffeine is more likely to inhibit than to promote cancer production by viral, physical or chemical agents. There is evidence to suggest that caffeine may have some anti-carcinogenic effects (p. 414).
Tomatis et al. [6]	Coffee and tea contain substances that are either direct mutagens (methylglyoxal) or which enhance the mutagenic effect produced by other chemicals (caffeine, theobromine) (p. 214).
IARC Monograph [7]	Coffee is possibly carcinogenic to the human urinary bladder. There is evidence suggesting lack of carcinogenicity of coffee drinking in the human female breast and in the large bowel. There is inadequate evidence in humans that coffee drinking is carcinogenic in the pancreas, ovary and other body sites. There is inadequate evidence in experimental animals for the carcinogenicity of coffee (p. 174). Research on the modifying effects of coffee on the activity of known mutagens and carcinogens is also limited to reveal any effect on tumor production (p. 92). It is noteworthy that coffee and tea, which have been consumed worldwide in large quantities for centuries, have been tested for carcinogenicity in experimental animals only recently (p. 37).
Rall [8]	The induction by caffeine of chromosomal abnormalities and mutagenic effects seems to be associated with inhibition of DNA-repair processes. They are observed only with concentrations of caffeine that are much in excess of those that follow the ingestion of beverages and medications (pp. 625–626).
Mohr et al. [9]	Coffee and caffeine, investigated for *in vivo* as well as *in vitro* carcinogenicity, cannot be definetely categorised as a mutagen or nonmutagen (p. 359). Results on the effects of caffeine in combination with known carcinogens vary from clear enhancement to clear inhibition of the occurrence of tumors (p. 359). On the whole, the results of the studies with various test systems on the enhancing or suppressing effects of caffeine on diverse mutagens/carcinogens do not permit any clear prediction (p. 374). Caffeine appears to enhance the effects of mutagens/carcinogens. The results of the mutagenicity studies may indicate that the main mechanisms of caffeine action involve its interaction with the repair of the damage caused either spontaneously or by mutagens/carcinogens in DNA and related structures. It is also possible that caffeine interacts with the enzymatic activation efficacy of promutagens/procarcinogens and other compunds (p. 374).
Ames and Gold [10, 11]	Over a thousand chemicals have been reported in roasted coffee: more than half of those tested (19/28) are rodent carcinogens. There are more rodent carcinogens in a single cup of coffee than potentially carcinogenic pesticide residues in the average American diet in a year, and there are still a thousand chemicals left to test in roasted coffee. This does not mean that coffee is dangerous but rather that animal cancer tests and worst-case risk assessment, build in enormous safety factors and should not be considered true risks.
Spiller [5]	It appears that both tea and coffee and other methylxanthine-containing products may be safe and may even be protective for certain cancers when consumed in moderation (pp. 339–340).

brewed coffee [2, 12, 13], our focus will be on caffeine; nonetheless, the main theses of the article are applicable in many respects to some of such products and to the many beverages resulting from coffee roasting and brewing.

The impact of caffeine on cell cycle checkpoint function

Caffeine can affect DNA repair, modify the apoptotic response and perturb cell cycle checkpoint integrity [4, 14–20]. Modification of p53 status by caffeine may interfere with normal induction of p53 in response to DNA damage [16].

Cells are acutely sensitive to broken DNA, and they employ fascinating mechanisms to regulate cell cycle progression and to insure DNA stability in the face of genotoxic stress [15, 21, 22]. Surveillance control mechanisms are hypothesised to give cells the ability to pause transiently during the cell cycle in response to agents that cause damage, particularly damage to DNA. These mechanisms are referred to as cell cycle checkpoints. They allow the cell time to arrest proliferation and repair damage; alternatively, the cell may undergo apoptosis (death) or enter an irreversible senescence-like state [23]. Key transitions in the cell cycle are tightly regulated by various protein kinase complexes composed of cyclin and cyclin-dependent kinases. A burgeoning area of research is addressing changes in G1, S phase (in which DNA replication occurs), and G2 checkpoint responses to double-strand DNA breaks when cells undergo genotoxic stress because of exposure to agents such as benzo[*a*]pyrene, ionizing radiation or ultraviolet (UV) radiation.

Caffeine and other methylxanthines can alter the G1, S and G2 checkpoint delay periods. When cells are treated with caffeine and DNA-damaging agents such as ionising radiation and alkylating agents, the lethality of the DNA-damaging agent is often potentiated. It has been known for many years that caffeine is involved in the sensitisation of DNA to damage: while advances in molecular biology nowadays enable an in-depth study at the molecular level, many chromosomal and cytological studies on the effects of caffeine were conducted over 25 years ago [1].

The molecular mechanisms of caffeine's varied actions remain to be fully elucidated. It does seem, though, that one of the consequences of the abrogation of the induction of the G1 delay following DNA damage is a failure to induce p53, and hence p21. Caffeine can also promote overriding of the G2/M block induced by irradiation. Caffeine may act as a radiosensitiser in cells with nonfunctional p53 activity. Such cells lack a checkpoint at G1/S and are more vulnerable to radiosensitisation because of the caffeine-induced abrogation of the G2/M checkpoint [24]. The variety of responses displayed by different cell types complicates the elucidation of the specific mechanism, especially as little is known about the capacity of caffeine to enhance DNA damage in normal, untransformed cells [25].

Much of the evidence on DNA repair processes is based on observations of the effects of caffeine in cells previously exposed to UV or to alkylating agents [1]. Specifically, caffeine has played an important role in gathering evidence about the lowered cell viability following DNA damage if G1 and G2 checkpoints are overridden. In turn, this evidence supports the notion that one function of the checkpoints systems is to allow cells time to stop to repair damage before continuing the cell cycle [23].

Similarly, correlations between reduced cell-cycle delays and increased sensitivity for killing – as for example, following caffeine treatment of irradiated cells – have also led to the conclusion that delays allow time for repair of potentially lethal damage [26]. Studies attempting to define a biochemical pathway for the induction of delay have focused on the action of caffeine and related xanthines and purines, prompted by the observation that caffeine sensitises bacteria to UV-induced cell killing. Mammalian cells are also radiosensitised by caffeine, and cells exposed to caffeine immediately postirradiation are not delayed in G2. It is possible that caffeine functions by inhibiting several phosphodiesterases and protein kinases like cyclic AMP phosphodiesterase, ATM or ATR [27, 28]. Experiments have used several caffeine-like agents, such as theophylline, theobromine and 2-aminopurine, whose shared activities include inhibition of protein kinases. It appears that caffeine not only blocks the expression of the delay, but also preserves the damage that gives rise to it, allowing the expression of delay on caffeine removal. Caffeine may thus sensitise cells to DNA damage by two mechanisms: (1) indirect inhibition of repair via abolition of checkpoint controls, and (2) direct inhibition of repair functions, perhaps by binding sites of damage. As with G2 delay, S-phase delay (or replicon initiation delay) is abolished by continuous caffeine treatment and postponed by finite treatments.

Several studies support the hypothesis that p53-mediated G1 delay following DNA damage may be abolished by caffeine. Studies also suggest the existence of a caffeine-susceptible checkpoint-control component that mediates delays in all three phases of interphase; such a component might be a protein-kinase [26]. Two important candidates for this caffeine checkpoint-inhibition are the protein-kinases ATM and ATR. When ATM and ATR detect double strand breaks they phosphorilate p53 and produce its dissociation from the negative regulator MDM2. p53 then undergoes further modification and activates transcription of genes responsible for cell cycle arrest. Under certain circumstances, p53 also activates transcription of genes responsible for apoptosis. The

dysfunction of this cascade of events is oncogenic, and the inactivation of ATM or ATR kinase is an alternative to p53 mutation [29]. On the other hand, two pathways seem to induce G2 arrest in response to DNA damage: one depends directly on p53, while the second is relatively p53-independent, but dependent on the protein-kinases ATM and ATR [30]. Thus, caffeine may also impede the G2 arrest indirectly or directly by the inhibition of ATM and ATR. Furthermore, in some *in vitro* experiments made in G1 checkpoint deficient cells, caffeine has produced premature chromatin condensation [31] (premature chromatin condensation is a hallmark of mammalian cells that begin mitosis before completing DNA replication; this lethal event is prevented by a highly conserved checkpoint involving a caffeine-sensitive mediator) [31].

The impact of caffeine on cell cycle checkpoint function occurs in spite of it being nonmutagenic in traditional mutagenesis assays. In this respect caffeine might resemble a number of chemicals found in the environment that do not show mutagenic properties in a variety of assays, yet affect cell surveillance, and may even have the ability to induce tumours in rodents [23]. It has been hypothesised that a nongenotoxic environmental carcinogen may function by ablating some aspect of cell cycle checkpoint function, perhaps leading to genetic instability or heritable alterations of the genome. The study of such environmental chemical agents may give insight into checkpoint signal transduction and mechanisms of carcinogenesis [23].

In summary, altering cell cycle checkpoint signalling pathways threatens DNA stability in the face of genomic stress, decreases cellular viability and increases cancer susceptibility. These processes are particularly clear in studies involving caffeine-induced 'checkpoint function over-ride' after DNA damage [23].

The metabolic impact of coffee, other caffeine-containing beverages, and other coffee-compounds

It has been known since long that caffeine increases the metabolic rate [8, 32]. Caffeine stimulates gastric secretion, gall bladder contraction and diuresis, among many other gastrointestinal and renal effects [2, 14, 33]. Methylxanthines augment release of the secretory products of a number of endocrine and exocrine tissues, including pancreatic hormone secretion [8]. It is unclear how these actions may affect absorption, distribution, storage and excretion of xenobiotics. Liver enzymes are also affected by caffeine [34, 35], and xenobiotics metabolised in the liver can thus be affected. Caffeine stimulates the production of cytochrome P450 (CYP) enzymes in the liver [36, 37]. It may thus interact with environmental substrates of CYP1A2, CYP1A1, CYP2E1, CYP3A or NAT2; many of such substrates are the object of epidemiologic studies on cancer causation [38–40]. One of the consequences may be an increase in the metabolic activation of promutagens like polycyclic aromatic hydrocarbons (PAHs) and aliphatic DNA-damaging compounds. However, the opposite effect may also occur in other instances, with caffeine decreasing the cytotoxic, cytostatic or mutagenic activity of aromatic DNA-damaging compounds, making stacking complexes with them, and decreasing the concentration of free aromatic procarcinogens available for cytochrome activation [41, 42].

Caffeine is an adenosine antagonist: it increases plasma adenosine concentration by an unknown mechanism, in a dose-dependent manner, at doses provided to humans by 3–6 cups of coffee per day [43]. Sudden changes in caffeine – and thus in plasma adenosine – concentrations can dramatically alter the physiology of many organ systems. As other methylxanthines, caffeine relaxes smooth muscle, notably bronchial muscle, and stimulates respiration. It also increases the conduction velocity of the heart and cardiovascular contractility, while it decreases peripheral vascular resistance. Such physiologic effects of caffeine may change the absorption of carcinogenic substances (i.e., the true 'exposure' of target tissues, as opposed to exposure reported by subjects or measured in their environment). Misclassification of exposure might hence be decreased if coffee consumption was taken into account.

In turn, many factors can affect the absorption, disposition and metabolism of caffeine. Caffeine is eliminated primarily by metabolism in the liver, through pathways involving at least CYP1A2, CYP1A1, CYP2E1, CYP3A, and NAT2. Obesity does not seem to affect much the pharmacokinetics of caffeine [44, 45]. By contrast, liver failure slows and cigarette smoking increases the clearance of caffeine [46]. Clearance is also enhanced by oral contraceptives, phenytoin, barbiturates and rifampin, among other drugs [8, 32]. Higher plasma and tissue concentrations may be achieved in the elderly than in younger individuals, and physiologic responses may also be greater in the elderly [47]. Again, the implication is that the effect modification that caffeine can cause may further vary by age, gender or physiologic state.

Agonistic or antagonistic interactions?

The direction of the interactions between coffee and agents present in the human environment (potentiation, antagonism, no effect) is difficult to predict. It is particularly hard to state whether coffee will enhance or abate the carcinogenic effect of chemical, physical or microbial agents; both enhancing and inhibitory effects have been reported in a large variety of experimental studies [1, 32]. The specific effect will depend on the environmental agent and setting; the physiologic, cellular or genetic alteration of interest;

the tissue or organ; and the conditions of administration, such as temperature and timing [1].

The toxicological and epidemiological interaction of coffee-drinking and smoking have been studied with some detail, and a dose-related inhibitory effect of coffee on the carcinogenic action of cigarette-smoke has been reported for some diseases, such as bladder cancer (Table 2 and Figure 1) [1, 48–63]. However, in addition to pharmacokinetic interactions, pharmacodynamic interactions should also be taken into account; some animal studies, for instance, suggest that caffeine consumption may be a contributing factor in the onset, maintenance of and relapse to tobacco dependence [64]. Other mechanisms whereby methylxanthines and smoking may contribute to carcinogenesis include their combined action on hormones, growth factors, homocysteine levels and neurotransmitters at the cellular level [65].

Few interactions between coffee and agents present in the occupational environment have been assessed. Many epidemiologic studies could contribute to generate evidence on this issue by reanalysing existing data sets.

Microbes, environmental residues and other chemicals in coffee

As many other agricultural commodities, coffee is susceptible to contamination by pesticides and by many other products used to improve crop yield, storage, transport and manufacture [66]. The species of coffee, and the methods of bean separation, roasting and brewing also substantially affect the chemical components of the beverage [13]. Hydroxyhydroquinone, a genotoxic intermediate metabolite

Table 2. Drinking coffee: can it modify the increase in bladder cancer risk caused by smoking?

Example A. An epidemiologic study assessed the effect of tobacco smoking on bladder cancer risk [48]. A 4-fold increased risk was found for current smokers. However, upon stratification by coffee-drinking it was seen that the risk associated with smoking was substantially higher among noncoffee drinkers than among regular coffee drinkers (Figure 1) [49]. Thus, drinking coffee may modify the increase in bladder cancer risk caused by smoking [50]. Today, an interesting body of biologic evidence explains why this may be so (see below).

Without a doubt, the importance of curtailing smoking is not diminished by the possibility that coffee may lower the risk of bladder cancer caused by tobacco. This view – from public health – is compatible with an interest – from a biologic-mechanistic angle – in epidemiologic and clinical studies of interactions [51].

Example B. An example of the many studies that assessed interactions between smoking and other exposures (in this case, with consumption of raw carrots), and that could have assessed the interaction between smoking and coffee, but did not, is the study by Pohlabeln et al. [52].

Example C. A study among Japanese males found that urinary cotinine was inversely related to urinary caffeine [53]. Smokers always showed lower urinary caffeine levels than nonsmokers (adjusting by coffee intake). Results support the notion that smokers eliminate caffeine faster [54]. Caffeine levels were barely influenced by NAT2, CYP1A1 and CYP2E1 polymorphisms, whereas they were clearly related to smoking [53].

In accordance with some other studies [55] (but not all), the mentioned study also found that coffee drinking increased the risk of bladder cancer only among nonsmokers [56].

All things considered, the epidemiologic finding on the coffee-smoking interaction upon bladder cancer risk [49] has remarkable biological coherence, whereas the biologic study of caffeine [53] has substantial epidemiological coherence [57, 58].

The relationship of smoking and bladder cancer may be stronger among slow acetylators than among rapid acetylators [59]. The causal role of regular coffee consumption within this causal web (beyond uses of caffeine for phenotyping) does not seem to have been established yet by epidemiologic studies.

Example D. As mentioned in the introduction, the main messages of this paper are applicable not only to carcinogenesis, but to other areas as well. Stanton and Gray found that relative risks for delayed conception were not increased for caffeine consumption among smoking women, while risks were increased in nonsmoking women with a high caffeine intake. The authors consider that the observed interaction supports the notion that smoking increases the rate of caffeine metabolism and that smoking cessation thus results in slower caffeine elimination [60, 61]. A similar interaction between caffeine and smoking, with caffeine affecting primarily nonsmokers, has been reported with regard to the increased risk of an early spontaneous abortion [62]. By contrast, maternal consumption of coffee was inversely associated with the risk of having a recognised Down syndrome pregnancy [63]; a significant interaction between coffee drinking and smoking was also observed: the inverse association remained only for nonsmoking mothers who drank four or more cups of coffee per day (OR: 0.48; 95% CI: 0.28, 0.82). According to the authors these results suggest that among nonsmoking mothers, high coffee consumption is more likely to reduce the viability of a Down syndrome conceptus than that of a normal conceptus [63].

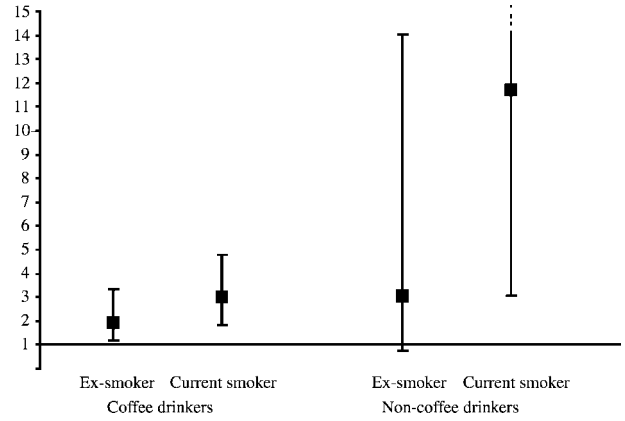

Figure 1. Coffee as an effect-modifier of the increase in the risk of bladder cancer caused by smoking. Odds ratios (and 95% confidence intervals) of bladder cancer for ex-smokers and for current smokers (compared with nonsmokers), stratified by coffee drinking [49].

of benzene, has been isolated in instant coffee [67], for instance.

Both bacteria and fungi have been found to degrade methylxanthines [1]. Ochratoxin A, a nephrocarcinogenic mycotoxin, has been found in improperly stored green coffee beans [68]; this toxin might be a contributory cause of testicular cancer [69].

Unfortunately, data on concentrations of environmental residues present in the coffee drank by individuals who take part in epidemiological studies is seldom available. Biological measurements of residues could be used along with data on the characteristics of coffee and of internal human dose. Standard questionnaires should continue to assess how coffee is prepared and consumed.

Finally, three points deserve to be stressed. Firstly, all over the world, different varieties of coffee beans are prepared in different ways; thus, the health effects observed by studies need not be uniform – on the contrary, they can be expected to be 'inconsistent'. Secondly, other chemical components of coffee (aliphatic, alicyclic, aromatic and heterocyclic compounds, carbohydrates, lipids, proteins, amino acids, alkaloids) can also have specific effects [2, 12, 13]; while some may be directly carcinogenic, others may enhance or inhibit the carcinogenic action of other agents [70]. Thirdly, in cultures where coffee (and tea) constitute a significant proportion of the total daily fluid intake, the mutually confounding effects of coffee drinking and total fluid intake should be considered [65].

Coffee drinking remains a potentially important causal factor

There are three main reasons that in our view explain why studies that aim at elucidating causal relationships relevant for the occurrence of cancer may benefit from considering coffee drinking as one of the causal factors.

- Firstly, as we saw, *caffeine exerts a large variety of physiological effects* (on the cardiovascular and central nervous systems, smooth muscles of the bronchi, skeletal muscle, kidney, etc.); these effects may affect the level of exposure to certain carcinogenic agents. For instance, as an ergogenic aid (it enhances endurance performance) [71], caffeine may indirectly increase the time of exposure to hazardous substances. By contrast, heavy coffee drinkers may have increased respiration and elimination of harmful occupational vapours. Because some coffee brewing techniques raise the serum concentration of total and low-density-lipoprotein cholesterol, the relationship between lipids and some cancers may be influenced by coffee lipids such as cafestol and kahweol, which are extracted by hot water but are retained by a paper filter [72, 73]. Females and nonsmokers may be at highest risk of experiencing the toxic effects of caffeine [74].
- Secondly, caffeine has significant cognitive, psychological and behavioural effects [2–5, 64, 75]; *these effects may also influence exposure to factors that can induce, promote or inhibit cancer* [76–79]. Smoking and alcohol drinking are often found to be higher among coffee drinkers [80, 81], while coffee abstainers have been found to eat both more [82] and less [83] fruits and vegetables than heavy coffee drinkers. We also found coffee consumption associated with higher education, lower level of physical activity, higher consumption of calories and saturated fat, and lower intake of carbohydrates, folates and vitamin C [80].
- Thirdly, from a knowledge-oriented perspective *gene–caffeine interactions* have an intrinsic interest, as mentioned earlier. Only recently have epidemiologic efforts been devoted to study gene–nutrient interactions, of which Kohlmeier et al. distinguish four types: nutrient regulation of gene transcription, dietary damage to DNA integrity, dietary enhancement of DNA integrity, and genetic susceptibility to nutrient-moderated disease [84]. Along these lines, Willett points out that hypotheses and supporting evidence relating dietary factors to cancer can be obtained from a variety of sources, including metabolic and biochemical studies; and he presents the example of the effect of diet on estrogen profiles, which in turn are thought to be related to some cancers [85, 86].

The paths ahead

Coffee-drinking and coffee-compounds should receive as much attention as other dietary components not only because of the previously mentioned mechanistic and methodologic reasons, but also because of

how widespread their exposure is. Approximately 80% of the world's population consumes caffeine [87], which is considered 'the world's most popular drug' [88]. There are not many man-elaborated products more widely used than tea and coffee. Often, in a given human group there are many more coffee-drinkers than smokers and than alcohol-drinkers, and their age-range is broad. The dose-range is also wide, with significant sections of the population drinking from 0 to 9 cups per day [2–5]. Thus, often the opportunity exists to assess dose-related effects within many different strata.

An intriguing epistemological – and practical – question is posed by the above-mentioned fact that interactions between caffeine-containing beverages and other environmental agents are difficult to predict from current knowledge. We know the *potential* for such products to modify the effect of many substances, but we often know less well or not at all which specific substances or groups of exposures will be affected and in what direction. This is so because biologic processes are often extremely complex. For instance, a single DNA damaging agent often generates more than one type of DNA damage; and in response to one type of DNA damage, several repair systems may act [21, 22]. There is a large number of substrates, competing exposures and gene products involved in many pathways. There are many compensatory, redundant, often robust mechanisms [89], and interactions may vary dramatically at low and at high doses of exposure [21–23]. The mechanistic complexity at the genetic, cellular and physiologic levels makes it hard to predict clinical and epidemiological effects. Conversely, interactions detected in epidemiologic studies often do not take into account intermediate events in a long causal chain; interactions may thus simply represent the joint effect of exposures occurring soon before the development of clinical disease [61].

Yet, clinical and epidemiological effects are observable. We may not always have the pertinent mechanistic explanation at hand, and seldom a detailed mechanistic explanation that expands across all the relevant biologic, physiologic and clinical levels. But effects observed in human beings in their usual living environment constitute highly relevant pieces of knowledge, if the observations have been attained through sound methods. (We here leave aside clinical and societal implications, and restrict ourselves to the generation of 'pure' biologic knowledge.)

Whether agonist or antagonist, expected or unexpected, biologically plausible or implausible, an interaction resulting only from 'data dredging' or a 'fishing expedition' is virtually always useless. But an interaction resulting from careful analysis of quality data from a large human group is often worth considering even if the mechanistic explanation is not complete, even if it is impossible to lay out in detail all the mechanistic pathways leading to the observed effect.

Of course, one must always be wary of an interaction that substantially challenges firmly established biologic knowledge, particularly if the latter cuts across several levels (cellular, clinical, etc.). This would, for instance, be the classic, well grounded example of the NAT2 polymorphism affecting the metabolism of isoniazid, by which slow acetylators are more susceptible to toxic effects and rapid acetylators are more likely to respond poorly [90, 91]. But such firmly integrated molecular, physiological and clinical evidence often does not exist. Some important pieces of the biologic puzzle are often missing [92]. The observed clinico-epidemiologic effect may be only partly compatible with some existing knowledge. Or it may even suggest some reasonable, albeit intriguing biologic hypothesis worth testing 'back' in the laboratory. This could be the case, for instance, of molecular epidemiological observations on caffeine and K-*ras* activation in exocrine pancreatic cancer (EPC) [92, 93]. In this neoplasm, mutations in the K-*ras* gene are deemed to provide an inappropriate drive for progression through G1. Interestingly, in EPC the frequency of K-*ras* mutations has been found to increase in a dose-related manner with increasing coffee consumption [94–97].

Observations of effect modification at the clinical and epidemiological levels, if derived from unbiased studies and consistently replicated, may constitute pieces of evidence of interest for biologic studies. Specifically, biologic studies may benefit from weighing to what extent their observations, explanations and causal judgements are plausible or coherent with the corresponding clinical and epidemiological observations [57]. With the necessary caveats, information on the use of caffeine-containing beverages should be integrated in studies on the role of gene–environment interactions in the pathogenesis of cancer.

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